

AMENDMENTS TO THE SPECIFICATION

Please make the following amendments to the specification.

Please replace paragraph 0313-0316 with the following paragraphs:

[0313] Fractionation was done by loading up to 500g per YM100 Amicon Microcon column (Millipore) followed by a 500g centrifugation for 40 minutes at 4C. Flow through "YM100"RNA consisting of about of the total RNA was used for library preparation or fractionated further by loading onto a YM30 Amicon Microcon column (Millipore) followed by a 13,500g centrifugation for 25 minutes at 4C. Flow-through "YM30" was used for library preparation as is and consists of less than 0.5% of total RNA. For the both the "ligation" and the "One-tailed" libraries, RNA was ~~dephosphorilated~~ dephosphorylated and ligated to an RNA (lowercase)-DNA (UPPERCASE) hybrid 5'-~~phosphorilated~~ phosphorylated, 3" idT blocked 3"-adapter (5"-P-uuuAACCGCATCCTTCTC-idT-3" (SEQ ID NO: 142673) Dharmacon # P-002045-01-05) (as elaborated in Elbashir et al., Genes Dev. 15:188-200 (2001)) resulting in ligation only of RNase III type cleavage products. 3"-Ligated RNA was excised and purified from a half 6%, half 13% polyacrylamide gel to remove excess adapter with a Nanosep 0.2M centrifugal device (Pall) according to instructions, and precipitated with glycogen and 3 volumes of Ethanol. Pellet was resuspended in a minimal volume of water.

[0314] For the "ligation" library a DNA (UPPERCASE)-RNA (lowercase) hybrid 5"-adapter (5"-TACTAATACGACTCACTaaa-3" (SEQ ID NO: 142674) Dharmacon # P-002046-01-05) was ligated to the 3"-adapted RNA, reverse transcribed with "EcoRI-RT": (5"-GACTAGCTGGAATTCAAGGATGCGGTAAA-3") (SEQ ID NO: 142675), PCR amplified with two external primers essentially as in Elbashir et al 2001 except that primers were "EcoRI-RT"and "PstI Fwd"(5"-CAGCCAACGCTGCAGATACGACTCACTAAA-3") (SEQ ID NO: 142676). This PCR product was used as a template for a second round of PCR with one hemispecific and one external primer or with two hemispecific primers.

[0315] For the "One tailed" library the 3"-Adapted RNA was annealed to 20pmol primer "EcoRI RT" by heating to 70C and cooling 0.1C/sec to 30C and then reverse transcribed with Superscript II RT (According to instructions, Invitrogen) in a 20l volume for 10 alternating 5 minute cycles of 37C and 45C.

Subsequently, RNA was digested with 1l 2M NaOH, 2mM EDTA at 65°C for 10 minutes. cDNA was loaded on a polyacrylamide gel, excised and gel-purified from excess primer as above (invisible, judged by primer run alongside) and resuspended in 13l of water. Purified cDNA was then oligo-dC tailed with 400U of recombinant terminal transferase (Roche molecular biochemicals), 1l 100M dCTP, 1l 15mM CoCl₂, and 4l reaction buffer, to a final volume of 20l for 15 minutes at 37°C. Reaction was stopped with 2l 0.2M EDTA and 15l 3M NaOAc pH 5.2. Volume was adjusted to 150l with water, Phenol : Bromochloropropane 10:1 extracted and subsequently precipitated with glycogen and 3 volumes of Ethanol. C-tailed cDNA was used as a template for PCR with the external primers "T3-PstBsg(G/I)18"(5"-AATTAACCCTCACTAAAG GCTGCAGGTGCAGGTGGGTTGGGTTGGGTTGN-3") (SEQ ID NO: 142677) where I stands for Inosine and N for any of the 4 possible deoxynucleotides), and with "EcoRI Nested"(5"-GGAATTCAAGGATGCGGTTA-3") (SEQ ID NO: 142678). This PCR product was used as a template for a second round of PCR with one hemispecific and one external primer or with two hemispecific primers.

[0316] Hemispecific primers were constructed for each predicted GAM RNA oligonucleotide by an in-house program designed to choose about half of the 5" or 3" sequence of the GAM RNA corresponding to a TM of about 30-34C constrained by an optimized 3" clamp, appended to the cloning adapter sequence (for "One-tailed" libraries 5"-GGNNGGGNNG (SEQ ID NO: 142679) on the 5" end of the GAM RNA , or TTAAACCGCATC-3" (SEQ ID NO: 142680) on the 3" end of the GAM RNA. For "Ligation" libraries the same 3" adapter and 5"-CGACTCACTAAA (SEQ ID NO: 142681) on the 5" end). Consequently, a fully complementary primer of a TM higher than 60C was created covering only one half of the GAM RNA sequence permitting the unbiased elucidation by sequencing of the other half.

Please replace 0323 with the following paragraph:

Reference is now made to Fig. 23B, which is a schematic representation of secondary folding of each of the MIRs and GAMS of GR GRI2731 MIR24 (SEQ ID NO: 142697), MIR23 (SEQ ID NO: 142690), GAM22 (SEQ ID NO: 142691) and GAM116 (SEQ ID NO: 142692), and of the negative control non-GAM hairpins, herein designated N2 (SEQ ID NO: 142688), N3 (SEQ ID NO: 142689), N116 (SEQ ID NO: 142693), N4 (SEQ ID NO: 142694), N6 (SEQ ID NO: 142696) and N7 (SEQ ID NO: 142698). N0 (SEQ ID NO: 142695) is a non-hairpin control, of a similar length to that of known MIR PRECURSOR hairpins. It

is appreciated that the negative controls are situated adjacent to and in between real MIR genes and GAM predicted oligonucleotide and demonstrates similar secondary folding patterns to that of known MIRs and GAMS.

Please replace paragraph 0328 with the following paragraph:

Reference is now made to Fig. 24A which is an annotated sequence of an EST comprising a novel GAM oligonucleotides detected by the oligonucleotide detection system of the present invention. Fig. 24A shows the nucleotide sequence of a known human non-protein coding EST (Expressed Sequence Tag), identified as EST72223 (SEQ ID NO: 142699). The EST72223 clone obtained from TIGR database (Kirkness and Kerlavage, 1997) was sequenced to yield the above 705bp transcript with a polyadenyl tail. It is appreciated that the sequence of this EST comprises sequences of one known miRNA oligonucleotide, identified as hsa-MIR98, and of one novel GAM oligonucleotide referred to here as GAM25, detected by the BIOINFORMATIC OLIGONUCLEOTIDE DETECTION ENGINE 100 (Fig. 9) of the present invention.

Please replace paragraph 0337 with the following paragraph:

Transcript products were 705nt (EST72223), 102nt (MIR98 precursor), 125nt (GAM25 precursor) long. EST72223 was PCR amplified with T7-EST 72223 forward primer: 5'-TAATACGACTCACTATAGGCCCTTATTAGAGGATTCTGCT-3' (SEQ ID NO: 142682) and T3-EST72223 reverse primer: 5'-AATTAACCCTCACTAAAGGTTTTTTTTCCTGAGACAGAGT-3' (SEQ ID NO: 142683). MIR98 was PCR amplified using EST72223 as a template with T7MIR98 forward primer: 5'-TAATACGACTCACTATAGGGTGAGGTAGTAAGTTGTATTGTT-3' (SEQ ID NO: 142684) and T3MIR98 reverse primer: 5'-AATTAACCCTCACTAAAGGGAAAGTAGTAAGTTGTATAGTT-3' (SEQ ID NO: 142685). GAM25 was PCR amplified using EST72223 as a template with GAM25 forward primer: 5'-GAGGCAGGAGAATTGCTTGA-3' (SEQ ID NO: 142686) and T3-EST72223 reverse primer: 5'-AATTAACCCTCACTAAAGGCTGAGACAGAGTCTTGCTC-3' (SEQ ID NO: 142687).